United States Department of Agriculture

Food Safety and Inspection Service

Office of Public Health Science

Science Staff

The Nationwide Microbiological Baseline Data Collection Program: Raw Liquid Eggs Survey

March 2012 – February 2013

FOREWORD

This report provides an overview of The Nationwide Microbiological Baseline Data Collection Program: Raw Liquid Eggs Survey. The Microbiological Analysis and Data Staff, Science Staff, Office of Public Health Science conducted this survey during a 12-month period (March 2012 to February 2013) to estimate the *Salmonella* percent positive and indicator bacteria levels in raw liquid eggs. FSIS used this information to estimate national prevalence of *Salmonella* in whole eggs, egg whites, and egg yolks. FSIS inspection program personnel in the Office of Field Operations (OFO) collected samples, which were analyzed by Food Safety Net Services, Ltd., San Antonio, TX, via a contract.

The Nationwide Microbiological Baseline Data Collection Program: Raw Liquid Eggs BASELINE Survey (RLEBS)

MARCH 2012 – FEBRUARY 2013

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The Nationwide Microbiological Baseline Data Collection Program:

Raw liquid eggs BASELINE Survey (RLEBS)

March 2012 – February 2013

EXECUTIVE SUMMARY

FSIS conducted the Raw Liquid Egg Baseline Survey (RLEBS) from March 2012 to February 2013 to determine the presence and levels of bacteria in certain raw liquid egg products prior to Pasteurization The survey generated a total of 1,853 samples from three primary liquid egg product types. Liquid egg products – 726 whole egg samples, 573 egg white samples, and 544 egg yolk samples - were collected at pre-Pasteurization from 48 eligible establishments that produce liquid eggs under federal inspection. All Federal establishments producing liquid egg products that were identified during shakedown were included in the sampling frame and were eligible for selection during the actual survey. The number of samples collected from each establishment was based on volume and ranged from 3 to 95, with an average of 39 samples per establishment. These samples were analyzed to determine the percent positive and pathogen levels for *Salmonella*, as well as the levels for generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, and total coliforms. FSIS compared percent positive and levels of specific microbiological targets to determine if significant differences existed between samples taken from different production shifts.

Summary findings

The percentage positives for *Salmonella* were 34.1% for whole eggs, 8.3% for egg whites, and 26.1% for egg yolks. Because of an observed large variation in lot sizes, when adjusted for this variation the percentage positives for Salmonella were 43.1% for whole eggs, 10.6% for egg whites, and 31.9% for egg yolks.

FSIS compared levels in each liquid egg product for *Salmonella,* generic *E. coli,* and APC based on production in first versus second production shifts. No statistically significant difference was observed for any pathogen or indicator bacteria in any of the three liquid egg products.

The top three most frequent *Salmonella* serotypes found in each product were:
Whole Eggs: Heidelberg (30.0%), Braenderup (24.7%), and Enteritidis (11.6%).
Egg Whites: Braenderup (20.8%), Kentucky (20.8%), and Heidelberg (14.6%).
Egg Yolks: Enteritidis (23.4%), Kentucky (20.7%), and Heidelberg (19.3%).

FSIS calculated the prevalence or weighted average of *Salmonella* for samples of each liquid egg product type. These national prevalence estimates are different from the percent positives because they are weighted in relation to production volume. The estimated prevalence of *Salmonella* in whole egg is **41.06%** with a 95% confidence interval between 18.78% and 63.33%. The estimated prevalence of *Salmonella* in egg white is **6.99%** with a 95% confidence interval between 2.44% and 11.54%. The estimated prevalence of *Salmonella* in egg yolk is **27.21%** with a 95% confidence interval between 10.36% and 44.07%. FSIS regulatory testing programs have consistently reported a very low incidence for *Salmonella* in Pasteurized products, suggesting that the presence of *Salmonella* in raw liquid egg products pre-Pasteurization does not necessarily represent an issue for public health. Additionally, the findings reported here should not be extended to what might be detected in shell eggs when tested for the same microbial targets.

INTRODUCTION

The United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), is responsible for the enforcement of the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act. These Acts empower the Agency to inspect raw and processed meat, poultry, and egg products for evidence of adulteration and exposure to insanitary conditions. In addition, using provisions cited under these Acts, the Secretary of Agriculture is authorized to promote special assessments, such as baseline surveys, to estimate the presence (qualitative levels) and number (quantitative levels) of pathogens and indicator bacteria in raw products. Baseline surveys are statistically designed to assess the industry as a whole by weighting sampling of each establishment according to its relative production volume. Because the data is weighted by production volume, quantitative pathogen data from this and other baseline studies provide a scientific basis for exposure assessment used in microbial risk assessments. The baseline survey establishes microbiological profiles of sampled commodities and determines liquid egg production parameters in the prevalence and levels of pathogen and indicator bacteria. Data collected during baseline surveys is essential for meeting these mission-critical needs.

FSIS conducted the Raw Liquid Egg Baseline Survey (RLEBS) for 12 consecutive months from March 2012 to February 2013. The RLEBS testing included three specific liquid egg products – whole eggs, egg whites, and egg yolks, which were the products recommended for testing by an agency working group. Microbiological test data generated from testing these three egg products are used to determine FSIS performance standards for liquid egg products. A 90-day training period preceded the survey (shakedown) for the field and laboratory personnel to prepare for the baseline. In addition, FSIS created a mailbox system for inspection program personnel (IPP) to submit questions and obtain information about the survey. FSIS also utilized formal FSIS Notices providing survey information and instructions for sampling.

OBJECTIVES

The Liquid Egg Baseline Survey had six objectives:

1. Collect microbiological data from Liquid Egg - whole egg, egg white, and egg yolk samples - to determine the presence and quantitative levels of specific microbiological targets and measure the change over time. Microbiological targets included:

   Pathogen:
   - *Salmonella*

   Indicator bacteria:
   - Generic *Escherichia coli*
   - Total Aerobic Plate Count (35 ^{o}C APC)
   - Enterobacteriaceae
   - Total coliforms

2. Calculate the national prevalence of *Salmonella* in each liquid egg product using the production volume as a reference for weighting the samples. The RLEBS will account for nonresponses if necessary.

3. Provide data to support the development of industry performance guidance for pathogen contamination of each egg product.

4. Provide data for use in the development of risk assessments, which inform risk management decisions, risk-based sampling programs, and regulatory policy decision making.

5. Obtain *Salmonella* isolates to generate serotyping data.

PROGRAM DESIGN

Establishments Included in the Sampling Frame

FSIS's computerized database, the Public Health Information System (PHIS), does not contain information on egg products production volume or establishments producing egg products. During the shakedown period, FSIS surveyed all establishments that produce raw liquid egg products, obtaining the production volume for these establishments. This information allowed the Agency to categorize eligible establishments for the sampling frame according to production volume and to allocate them to the three corresponding strata. During the actual survey, production volume information for each establishment was updated with new production results periodically. All Federal establishments producing liquid egg products that were identified during this phase were included in the sampling frame and were eligible for selection during the actual survey (17).

FSIS identified 56 establishments producing any form of raw quid egg product. These establishments were eligible for the baseline survey and represented all liquid egg products produced in the United States under Federal inspection

Sample Collection Design

The sample design considered establishment production volume, the nature and number of bacterial targets, logistical sampling limitations, the specific data to be collected, sampling costs, and the collection and analytical methods.

Two types of errors were considered—sampling errors and nonsampling errors. Both sampling and nonsampling errors may affect the reliability of results and had to be considered in designing this survey. Sampling errors occur because observations are derived from a subset of the entire population; nonsampling errors may be attributed to many sources inherent in the collection of samples, laboratory analysis, and processing of data. FSIS considered these types of errors in determining the total sample size and the number of samples to be collected from each establishment.

The RLEBS incorporated a multi-stage cluster design that included sampling each establishment over time. Each liquid egg product sample was selected at frequencies defined by three production volume categories or strata. Strata based on the production volume accrued during the actual 12-month survey were used for sampling weight and calculation of prevalence. These strata do not correlate to PR/HACCP establishment size.

For Whole Eggs:
Production Volume Category 1 consisted of large establishments that produce more than 40,000,000 pounds of liquid whole egg per year. This stratum contains establishments that produce 71.7% of the total whole egg in the sampling frame[1].

Production Volume Category 2 consisted of medium establishments that produce 8,300,000 or more pounds of whole egg, but fewer than 40,000,000 pounds per year. This stratum contains establishments producing 26.7% of the total whole egg in the sampling frame.

Production Volume Category 3 consisted of small establishments that produce less than 8,300,000 pounds of whole egg per year. This stratum contains establishments producing 1.6% of the total whole egg in the sampling frame.

[1] In statistics, a sampling frame is the source from which samples may be drawn (eligible establishments). The sampling frame is composed of the establishments that may be sampled; in this survey there are 3 sampling frames, one for each liquid egg product.

For Egg White:
Production Volume Category 1 consisted of large establishments that produce more than 20,000,000 pounds of liquid egg white per year. This stratum contains establishments that produce 65.5% of the total egg white in the sampling frame.

Production Volume Category 2 consisted of medium establishments that produce 6,000,000 or more pounds of egg white, but fewer than 20,000,000 pounds per year. This stratum contains establishments producing 30.1% of the total egg white in the sampling frame.

Production Volume Category 3 consisted of small establishments that produce less than 6,000,000 pounds of egg white per year. This stratum contains establishments producing 4.4% of the total egg white in the sampling frame.

For Egg Yolk:
Production Volume Category 1 consisted of large establishments that produce more than 11,000,000 pounds of liquid egg yolk per year. This stratum contains establishments that produce 69.7% of the total egg yolk in the sampling frame.

Production Volume Category 2 consisted of medium establishments that produce 2,900,000 or more pounds of egg yolk, but fewer than 11,000,000 pounds per year. This stratum contains establishments producing 26.0% of the total egg yolk in the sampling frame.

Production Volume Category 3 consisted of small establishments that produce less than 2,900,000 pounds of egg yolk per year. This stratum contains establishments producing 4.3% of the total egg yolk in the sampling frame.

To ensure sample distribution between shifts, sample collectors were instructed to alternate sample collection between Shift 1 and Shift 2 in those establishments that produced liquid egg during two production shifts.

Sampling Location within the Establishment

Inspectors collected raw liquid egg samples from the balance tank or, if not available, from an alternative location at the storage silo/tank sampling port, or at the collection pot during the egg breaking process. These are the points of collection in the establishment before Pasteurization of the liquid egg product. FSIS collected samples throughout the 12-month survey from these locations in the production line and from multiple production shifts in establishments with two shifts.

Sample Collection and Description

Samples were collected aseptically by FSIS inspectors following the procedures described in FSIS Notice 16-12, instructions provided on the computer-generated sample forms, and specific program directives. Inspectors collected about 400 ml of liquid egg product and placed it into a 500 ml sterile screw-capped plastic sample jar. Inspectors collected samples of:

- Whole egg
- Egg white, and
- Egg Yolk

To accommodate all sample requirements, inspectors were instructed to follow the sample collection procedures included in Section VIII, Baseline Sampling Procedures of the FSIS Notice 16-12 (Appendix).

The samples were shipped to the contract laboratory by an overnight delivery service on the same day that they were collected, or the next day if the sample was collected on the second shift. The samples were collected Monday through Friday during regular establishment operating hours (Monday through Thursday for second shift). Only those samples received at the laboratory the day after the sample was shipped with a

sample receipt temperature between 1°C to 10°C (inclusive) were analyzed. FSIS discarded samples received outside this temperature range or for other reasons such as leaking containers.

SELECTION OF MICROORGANISMS

To obtain microbiological data for use in the development of risk assessments, risk-based sampling programs, and regulatory policy decision making, the samples were analyzed for *Salmonella* (pathogen), and for indicator bacteria including generic *Escherichia coli*, total coliforms, 35˚C Aerobic Plate Count (APC), and Enterobacteriaceae (EB).

SAMPLE ANALYSIS METHODS

Indicator Bacteria

Liquid egg product samples were analyzed for Enterobacteriaceae (*1*), generic *E. coli* and coliforms (*2*) and Aerobic Plate Count (*3*) using 3M™ Petrifilm™. Samples were diluted 1:10 in Butterfields Phosphate Diluent and plated to represent the 10^{-1} dilution. Serial dilutions were prepared and plated to the 10^{-4} dilution. Each dilution was plated in duplicate as described in FSIS Microbiology Laboratory Guidebook (MLG) Chapter 3.01 for indicator bacteria (*4*). The Limit of Detection (LOD) for all indicator testing was 10 CFU/ml of egg product.

Salmonella

Liquid egg samples were analyzed for the presence of *Salmonella* using FSIS MLG methodology (*4*). A test portion of 100 ml of product was diluted 1:10 in Buffered Peptone Water (BPW), mixed, and incubated. The following day, enrichments were screened for *Salmonella* using MLG 4C.02 and the DuPont Qualicon BAX system (*5*). Samples with screen-positive results were culture-confirmed to contain *Salmonella* spp. using MLG 4.04). Following a screen-positive result, quantitative testing was initiated using refrigerated sample reserves. The theoretical LOD for qualitative detection of *Salmonella* was one cell per test portion or 0.01 CFU/ml of egg product.

The level of *Salmonella* in the screen-positive samples was estimated using the "Most Probable Number" (MPN) procedure (*6*). MPN analyses were initiated on the second day of analysis as soon as qualitative screening results were known. Three 10ml, three 1ml and three 0.1ml samples were analyzed using the previously described MLG methods. For some samples, additional dilutions were tested. The pattern of positive and negative results among these individual qualitative tests was used to statistically estimate low levels of *Salmonella*, and the results were expressed as "MPN/ml" of egg product. The presence of *Salmonella* in the positive tubes was culture-confirmed by the FSIS MLG method. Those *Salmonella* MPN results where at least one tube was positive for *Salmonella* were labeled as "quantifiable" samples in the data tables of this report. The theoretical LOD for the quantitative procedure was 0.03 MPN/ml of egg product.

DATA ANALYSIS METHODS

General Overview

The national prevalence calculation estimates *Salmonella* in raw liquid egg. National prevalence provides a national average of expected values for *Salmonella* in raw liquid egg products. Sampling was designed so the results represent all establishments manufacturing liquid egg products in the US. This approach uses class or "strata" by production volume for each egg product and defines the strata as large, medium, and small. This design ensures that small establishments are adequately represented in the study despite their low production volume. However, strata sampling introduces bias in the sample collection. To counterbalance this bias, all establishments were weighted using the total national production during the 12 months of the survey. After these considerations, the specialized statistical software WesVar v 5.1 (7) was used to calculate the national

prevalence estimate and its uncertainty. Details of file development, sample weight calculation, and software processing are presented in the appendix (Statistical Analysis Methods).

Results

RLEBS produced 1,853 valid samples: 726 for whole egg; 573 for egg white; 554 for egg yolk. In establishments that operated and processed liquid egg products during two shifts, samples were collected during both shifts. Inspectors collecting samples alternated their collection activities between the two shifts. As a result, the samples were approximately evenly distributed between both shifts.

Inspectors withdrew samples from large tanks containing different amounts of liquid egg. If a sample was *Salmonella* positive, the assumption was that because of mixing and distribution within the tanks, the entire volume of liquid egg product contained in the tank was positive. Because the tanks contain different volumes of liquid egg product, the percent positive based on the sample result alone does not exactly represent the percent positive of the volume of liquid egg contaminated. There was a need to adjust the percent positive to account for the lot-sized tank that the sample represents. In other words, the percent positive will be the addition of the volume of egg that *Salmonella* contaminated in the tanks divided by the addition of all tanks tested. In mathematical terms, if a *Salmonella* sample result (R) is a positive sample will make R = 1, and if results produce a negative sample, then R = 0. In addition, if the weight of a tank is W_i given by

$$W_i = V_i / \sum V_i$$

Where:

V_i is the volume of tank *"i"* and
$\sum V_i$ is the addition of the volume of all tanks tested

The adjusted per lot-size percent positive PP_{adj} is $PP_{adj} = \sum R\, W_i$ for all valid samples

Using the above equation and the data-files, the *Salmonella* percent positive and the adjusted by lot-size percent positives are shown in the following table by egg product:

Product	Percent Positive from samples	Percent Positive Adjusted by lot-size
Whole Egg	34.1	41.1
Egg White	8.3	10.6
Egg Yolk	26.1	31.9

All adjusted by lot-size percent positives are higher than the sample-based percent positives. The differences indicate that larger lots, which have more weight, are more predisposed to be positive than smaller lots. A plausible explanation is that, the more eggs are in the lot, the probability of having at least one contaminated egg affecting the entire lot is higher.

In addition, the percent of positive *Salmonella* samples of whole eggs corresponds almost exactly with the percent of its two components - egg white and egg yolk (34.1 ≈ 8.3 + 26.1). This seems to indicate that at separation there is no cross-contamination between white and yolk and each component is inherently about 8.3% *Salmonella* positive for whites and 26.1% for yolk. It also suggests that yolk supports the survival and growth of pathogens more than egg white.

Given that each liquid egg product has different characteristics, the statistical results and tables are shown in three separate sections:

A) Whole Eggs
B) Egg Whites, and
C) Egg Yolks

A) Whole Eggs

Table A1 presents a summary of the quantitative sample test results. For raw whole egg, 99.86% of the APC samples were above the limit of detection (LOD) (LOD <10 CFU/ml) for these microorganisms, while 92.84% of the samples were above the LOD for Enterobacteriaceae. The percent of samples above the LOD for total coliforms and generic *E. coli* were 88.29% and 62.71%, respectively.

Tables of the distribution of pathogenic microorganism levels were assembled for *Salmonella* (Table A2). The distribution of indicator organisms is detailed for APC (Table A3), Enterobacteriaceae (Table A4), Total Coliforms (Table A5), and generic *E. coli* (Table A6). These distributions are presented in ranges of factors of 10.

For *Salmonella* serotyping, the sample's prevailing colony was picked and processed. The results do not necessarily represent the only serotype in the sample. The *Salmonella* serotypes isolated most often in raw whole egg samples were Heidelberg (30.0%), Braenderup (24.7%), and Enteritidis (11.6%). Table A7 shows the frequencies and percentages calculated for the dominant *Salmonella* serotypes.

FSIS performed a comparison of the average presence of the microorganisms at Shift 1 to Shift 2, identifying 22 establishments with two shifts for this comparison. Two indicators, generic *E. coli* and APC, and the pathogen *Salmonella*, were selected for the comparison. A Goodness-of-Fit test was conducted on the data to see if the source distribution was normal. The Shapiro-Wilk "W" test rejected the hypothesis of normality at p> 0.0001 for all distributions; consequently, a non-parametric Wilcoxon/Kruskal-Wallis test (Rank Sums) was conducted in all cases (8). Table A8 details this comparison, which indicates no significant difference between Shifts 1 and 2 for all microorganisms.

FSIS calculated the prevalence or weighted average in relation to production volume for *Salmonella*. This national prevalence estimate is different from the percent positive, because it is weighted in relation to production volume and adjusted for nonresponse. Figure A1 shows the WesVar software output window for *Salmonella* prevalence in raw whole eggs. In summary, the results of prevalence are:

The estimated prevalence of *Salmonella* in raw whole eggs is 41.06% with a 95% confidence interval between 18.78% and 63.33%.The survey results for *Salmonella* prevalence differed from the percent positive rates for whole eggs and the other egg products. These differences exist because of the introduction of production volume weighting necessary to compensate for the survey's design bias.

B) Egg Whites

Table B1 presents a summary of the test results of all quantified samples. For raw egg white, 99.13% of the APC samples were above the limit of detection (LOD) (LOD <10 CFU/ml) for these microorganisms, while 90.23% of the samples were above the LOD for Enterobacteriaceae. The percentages of samples above the LOD for total coliforms and generic *E. coli* were 82.72% and 51.66%, respectively.

Tables for the distribution of pathogenic microorganism levels were assembled for *Salmonella* (Table B2). The distributions of indicator organisms are detailed for APC (Table B3), Enterobacteriaceae (Table B4), Total Coliforms (Table B5), and generic *E. coli* (Table B6). These distributions are presented in ranges of factors of 10.

For *Salmonella* serotyping, the sample's prevailing colony was picked and processed. The results do not necessarily represent the only serotype in the sample. The *Salmonella* serotypes isolated most often in egg white samples were Braenderup (20.8%), Kentucky (20.8%), and Heidelberg (14.6%). Table B7 shows the frequencies and percentages calculated for the dominant *Salmonella* serotypes.

FSIS performed a comparison of the average presence of the microorganisms at Shift 1 to Shift 2, identifying 22 establishments with two shifts for this comparison. Two indicators, generic *E. coli* and APC, and the pathogen *Salmonella*, were selected for the comparison. A Goodness-of-Fit test was conducted on the data to see if the source distribution was normal. The Shapiro-Wilk "W" test rejected the hypothesis of normality at p> 0.0001 for all distributions; consequently, a non-parametric Wilcoxon/Kruskal-Wallis test (Rank Sums) was conducted

in all cases (8). Table B8 details this comparison, which indicates no significant difference between Shifts 1 and 2 for all microorganisms.

FSIS calculated the prevalence or weighted average in relation to production volume for *Salmonella*. This national prevalence estimate is different from the percent positive, because it is weighted in relation to production volume and adjusted for nonresponse. Figure B1 shows the WesVar software output window for *Salmonella* prevalence in egg white samples. In summary the results of prevalence are:

The estimated prevalence of *Salmonella* in egg white is 6.99% with a 95% confidence interval between 2.44% and 11.54%. The survey results for *Salmonella* prevalence differed for the percent positive rates (*Salmonella*: percent positive of samples 8.3% versus national prevalence 6.9%).

C) Egg Yolks

Table C1 presents a summary of the test results of all quantified samples. For egg yolk, 97.29% of the APC samples were above the limit of detection (LOD) (LOD <10 CFU/ml) for these microorganisms, while 80.51% of the samples were above the LOD for Enterobacteriaceae. The percent of samples above the LOD for total coliforms and generic *E. coli* were 70.40% and 42.06%, respectively.

Tables for the distribution of pathogenic microorganism levels were assembled for *Salmonella* (Table C2). The distributions of indicator organisms are detailed for APC (Table C3), Enterobacteriaceae (Table C4), Total Coliforms (Table C5), and generic *E. coli* (Table C6). These distributions are presented in ranges of factors of 10.

For *Salmonella* serotyping, the sample's prevailing colony was picked and processed. The results do not necessarily represent the only serotype in the sample. The *Salmonella* serotypes isolated most often in egg yolk samples were Enteritidis (23.4%), Kentucky (20.7%), and Heidelberg (19.3%). Table C7 shows the frequencies and percentages calculated for the dominant *Salmonella* serotypes.

FSIS performed a comparison of the average presence of the microorganisms at Shift 1 to Shift 2, identifying 24 establishments with two shifts for this comparison. Two indicators, generic *E. coli* and APC, and the pathogen *Salmonella*, were selected for the comparison. A Goodness-of-Fit test was conducted on the data to see if the source distribution was normal. The Shapiro-Wilk "W" test rejected the hypothesis of normality at p> 0.0001 for all distributions; consequently, a nonparametric Wilcoxon/Kruskal-Wallis test (Rank Sums) was conducted in all cases (8). Table C8 details this comparison, which indicates no significant difference between Shifts 1 and 2 for all microorganisms.

FSIS calculated the prevalence or weighted average in relation to production volume for *Salmonella*. This national prevalence estimate differed from the percent positive because it is weighted in relation to production volume and adjusted for nonresponse. Figure C1 shows the WesVar software output window for *Salmonella* prevalence in egg yolk. In summary the results of prevalence are:

The estimated prevalence of *Salmonella* in egg yolk is 27.21% with a 95% confidence interval between 10.36% and 44.07%.The survey results for *Salmonella* prevalence differed from the percent positive rates (*Salmonella*: percent positive of samples 26.1% vs. national prevalence 27.2%.

DISCUSSION

Under the auspices of the Egg Products Inspection Act (21 U.S.C. 1031 *et seq.*), FSIS protects the health and welfare of consumers by preventing the distribution in commerce of egg products that are unwholesome, adulterated, or misbranded. The RLEBS was designed to determine the presence and the levels of selected bacteria in raw liquid egg products produced in federally inspected establishments. Accordingly, the findings reported here do not reflect what would be detected in shell eggs when tested for the same microbial targets.

A main goal of the survey was to calculate the national prevalence of *Salmonella* in each liquid egg product. Additional goals for the survey included determining if there was a significant difference between production shifts as it relates to bacterial levels and to provide a review of *Salmonella* serotypes found in raw liquid egg samples.

The source(s) of shell egg contamination which would influence the presence and levels of bacteria in raw liquid egg product include trans-ovarian transfer of certain serotypes, surface contamination of the egg shell by chicken feces, and contamination from the plant environment that is subsequently transferred to the raw liquid egg product (18, 20, 21, 22). While the percentage positive results may appear to be high for the pathogen *Salmonella*, these survey results are quite low when compared to similar studies (up to 92% of raw liquid egg samples positive for *Salmonella* [19]). Because these products are destined for Pasteurization, the presence and levels of *Salmonella* does not necessarily represent an issue for public health, since the most recent FSIS testing results indicated that the raw liquid eggs products tested in our regulatory program have an average percent positive rate of 0.14% for *Salmonella* in 2013 (23).

FSIS ability to produce a baseline on raw products will help to generate data that will be further analyzed to assist in the development of safe Pasteurization parameters, risk assessments, and other studies targeting specific issues.

TABLES AND FIGURES

SECTION A: WHOLE EGGS

Table A1. Summary for Quantified Whole Egg Samples by Microorganism

Microorganisms ---------- Indicator Organisms [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Levels of Positives Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count (APC)	Pre-Pasteurization	726	725	99.86%	421,516,021	347,376,904	15,068	(11,865 – 19,149)	4.17
Enterobacteriaceae	Pre-Pasteurization	726	674	92.84%	26,086	5,051	513	(412 – 640)	2.71
Total Coliforms	Pre-Pasteurization	726	641	88.29%	3,028	551	143	(121 – 170)	2.15
Generic *Escherichia coli*	Pre-Pasteurization	726	448	61.71%	844	187	25	(21 – 29)	1.39
Pathogenic Organism									
Salmonella [3]	Pre-Pasteurization	726	248 [4]	34.16%	4.47	0.66	0.35	(0.25 – 0.49)	-0.45

(1) Units are CFU/ml
(2) Above Limit of Detection (LOD); LOD = 10 CFU/ml
(3) *Salmonella* measurements are in MPN/ml, LOD < 0.030 MPN/ml
(4) There are 54 positives under LOD in the MPN test; LOD < 0.030 MPN/ml.

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Table A2. Distribution of *Salmonella* MPN Levels in Whole Egg Samples

Range, MPN/ml	Number of Samples[(1)]	Percent of Total	Cumulative Number	Cumulative Percent
< 0.030	54	21.8%	54	21.8%
0.030 - 0.30	85	34.3%	139	56.0%
0.301 - 3.00	43	17.3%	182	73.4%
3.01 - 30.00	64	25.8%	246	99.2%
> 30.01	2	0.8%	248	100.0%
Total	248	100.0%	-	-

LOD < 0.030 MPN/ml

(1) Only positive samples are included regardless if under LOD

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Table A3. Distribution of APC Levels in Whole Egg Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	1	0.1%	1	0.1%
10 - 100	34	4.7%	35	4.8%
101 - 1,000	116	16.0%	151	20.8%
1,001-10,000	204	28.1%	355	48.9%
10,001-100,000	170	23.4%	525	72.3%
100,001-1,000,000	139	19.1%	664	91.5%
1,000,001-10,000,000	44	6.1%	708	97.5%
10,000,001-100,000,000	7	1.0%	715	98.5%
100,000,001-1,000,000,000	6	0.8%	721	99.3%
> 1,000,000,000	5	0.7%	726	100.0%
Total	726	100.0%	-	-

LOD < 10 CFU/ml

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Table A4. Distribution of Enterobacteriaceae Levels in Whole Egg Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	52	7.2%	52	7.2%
10 - 100	199	27.4%	251	34.6%
101-1,000	197	27.1%	448	61.7%
1,001-10,000	148	20.4%	596	82.1%
10,001-100,000	98	13.5%	694	95.6%
100,001-1,000,000	29	4.0%	723	99.6%
> 1,000,000	3	0.4%	726	100.0%
Total	726	100.0%	-	-

LOD < 10 CFU/ml

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Table A5. Distribution of Total Coliforms Levels in Whole Egg Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	85	11.7%	85	11.7%
10 - 100	246	33.9%	331	45.6%
101-1,000	263	36.2%	594	81.8%
1,001-10,000	97	13.4%	691	95.2%
10,001-100,000	30	4.1%	721	99.3%
100,001-1,000,000	5	0.7%	726	100.0%
> 1,000,000	0	0.0%	726	100.0%
Total	726	100.0%	-	-

LOD < 10 CFU/ml

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Table A6. Distribution of Generic *Escherichia* Levels in Whole Egg Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
< LOD	278	38.3%	278	38.3%
10 -100	310	42.7%	588	81.0%
101-1,000	92	12.7%	680	93.7%
1,001-10,000	31	4.3%	711	97.9%
10,001-100,000	15	2.1%	726	100.0%
100,001-1,000,000	0	0.0%	726	100.0%
> 1,000,000	0	0.0%	726	100.0%
Total	726	100.0%	-	-

LOD < 10 CFU/ml

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Table A7. *Salmonella* Serotypes from Whole Eggs

***Salmonella* Serotypes**		
Serotype Name	**Count**	**Percentage**
Heidelberg	83	30.0%
Braenderup	34	24.7%
Enteritidis	34	11.6%
Kentucky	33	9.0%
Thompson	16	7.6%
Mbandaka	9	4.3%
Infantis	5	2.6%
Cerro	4	1.7%
4,5,12:i:-	2	1.4%
Johannesburg	2	1.1%
Montevideo	2	1.1%
Ohio	2	0.6%
Typhimurium var 5-	2	0.6%
Anatum	1	0.5%
Ealing	1	0.5%
Newport	1	0.3%
Rissen	1	0.3%
Schwarzengrund	1	0.2%
Tennessee	1	0.2%
Multiple Serotypes	14	0.2%
Total	**248**	**100%**

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Table A8. Statistical Comparison between Shift 1 and Shift 2 Whole Egg Samples from 22 Establishments

Indicators / Pathogen	Sample at Shift 1	Mean at Shift 1	Mean St Error at Shift 1	Geo Mean at Shift 1	Log10 of Geo Mean at Shift 1	Sample at Shift 2	Mean at Shift 2	Mean Sd Error at Shift 2	Geo Mean at Shift 2	Log10 Geo Mean at Shift 2	p-value (*)
Generic *E. coli* (CFU/ml)	217	998	278	39.33	1.59	164	1,999	686	35.56	1.55	p = 0.35
APC (CFU/ml)	217	8.7×10^6	5.9×10^6	23,932	4.37	164	1.4×10^8	1.4×10^8	23,990	4.38	p = 0.68
Salmonella (MPN/ml)	94	5.19	0.81	0.53	-0.27	66	6.57	2.06	0.57	-0.24	p = 0.90
Salmonella (Percent Positive)		43.31%					40.20%				p = 0.54**

(*) Test based on Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums) at α = 0.05 between shift 1 and shift 2 for actual values (22 establishments with 2 shifts)
(**) Test of Proportion for percent positive

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Table A9. Nonresponse Rate and Percent Positive by Strata for Whole Egg Samples

Strata	Discarded Samples	Valid Samples	Total Samples	Percent of Discarded	Percent Positive by Strata for *Salmonella*
1	39	226	265	14.7%	36.2%
2	48	447	495	9.7%	34.6%
3	52	53	105	49.5%	20.7%
Total/p-value	139	726	865	P= 0.001*	P = 0.005**

(*) p = 0.001 There is significant difference in percent discarded samples by strata.
(**) p = 0.005 There is significant difference in *Salmonella* percent positive by strata.

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Table A10. *Salmonella* Percent Positive by Month for Whole Egg Samples

Month Collected	Samples Collected	Samples *Salmonella* Positive	Percent Positive per Month
January	61	16	26.2%
February	63	20	31.7%
March	57	17	29.8%
April	49	18	36.7%
May	61	27	44.3%
June	60	23	38.3%
July	58	24	41.4%
August	67	25	37.3%
September	59	20	33.9%
October	67	25	37.3%
November	60	16	26.7%
December	64	17	26.6%

There is no statistical difference among percent positive per month (Test for homogeneity of proportions Chi-squared, p = 0.46)

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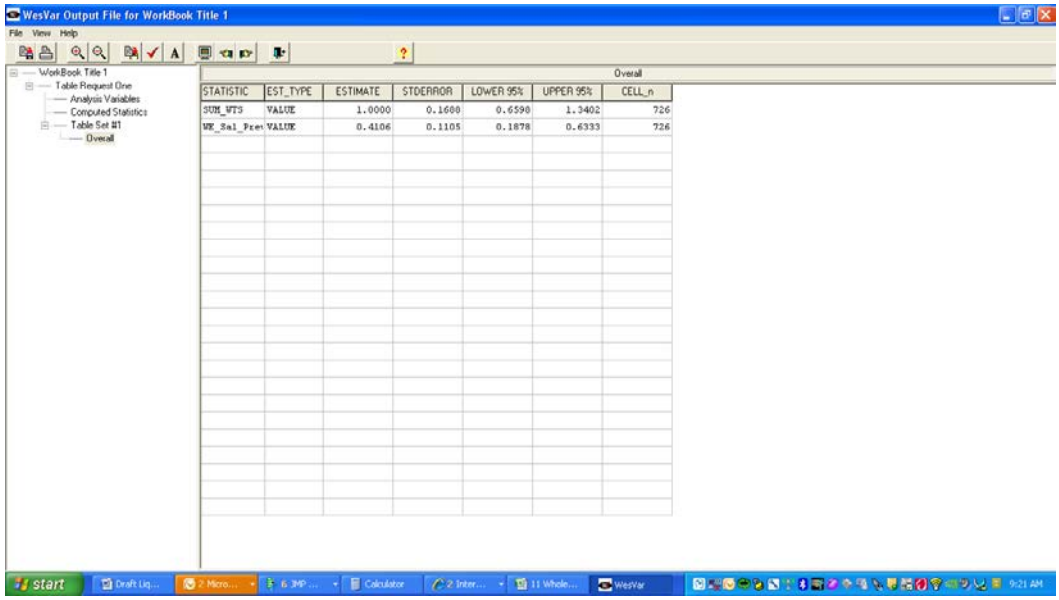
Figure 1A. WesVar Output Window for *Salmonella* Prevalence on samples of Whole Eggs.



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SECTION B: EGG WHITE

Table B1. Summary for Quantified Egg White Samples by Microorganism

Microorganisms ---------- Indicator Organisms [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Levels of Positives				
					Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count (APC)	**Pre-Pasteurization**	**573**	**568**	**99.13%**	**126,305**	**59,440**	**3,478**	**(2,843 – 4,255)**	**3.54**
Enterobacteriaceae	**Pre-Pasteurization**	**573**	**517**	**90.23%**	**2,506**	**486**	**151**	**(125 – 183)**	**2.18**
Total Coliforms	**Pre-Pasteurization**	**573**	**474**	**82.72%**	**1,145**	**551**	**52**	**(44 – 60)**	**1.71**
Generic Escherichia coli	**Pre-Pasteurization**	**573**	**296**	**51.66%**	**80**	**23**	**12**	**(10 – 14)**	**1.10**
Pathogenic Organism									
Salmonella [3]	**Pre-Pasteurization**	**573**	**48** [4]	**8.38%**	**3.57**	**0.87**	**0.26**	**(0.12 – 0.58)**	**-0.57**

(1) Units are CFU/ml
(2) Above Limit of Detection (LOD); LOD = 10 CFU/ml
(3) *Salmonella* measurements are in MPN/ml, LOD < 0.030 MPN/ml
(4) There are 13 positives under LOD in the MPN test; LOD < 0.030 MPN/ml.

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Table B2. Distribution of *Salmonella* MPN Levels in Egg White Samples

Range, MPN/ml	Number of Samples[(1)]	Percent of Total	Cumulative Number	Cumulative Percent
< 0.030	13	27.1%	13	27.1%
0.030 - 0.30	15	31.3%	28	58.3%
0.301 - 3.00	8	16.7%	36	75.0%
3.01 - 30.00	12	25.0%	48	100.0%
> 30.01	0	0.0%	48	100.0%
Total	48	100.0%	-	-

LOD < 0.030 MPN/ml

(1) Only positive samples are included regardless if under LOD

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Table B3. Distribution of APC Levels in Egg White Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	5	0.9%	5	0.9%
10 - 100	30	5.2%	35	6.1%
101 - 1,000	154	26.9%	189	33.0%
1,001-10,000	196	34.2%	385	67.2%
10,001-100,000	136	23.7%	521	90.9%
100,001-1,000,000	44	7.7%	565	98.6%
1,000,001-10,000,000	7	1.2%	572	99.8%
10,000,001-100,000,000	1	0.2%	573	100.0%
100,000,001-1,000,000,000	0	0.0%	573	100.0%
> 1,000,000,000	0	0.0%	573	100.0%
Total	573	100.0%	-	-

LOD < 10 CFU/ml

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Table B4. Distribution of Enterobacteriaceae Levels in Egg White Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	56	9.8%	56	9.8%
10 - 100	212	37.0%	268	46.8%
101-1,000	198	34.6%	466	81.3%
1,001-10,000	75	13.1%	541	94.4%
10,001-100,000	30	5.2%	571	99.7%
100,001-1,000,000	2	0.3%	573	100.0%
> 1,000,000	0	0.0%	573	100.0%
Total	573	100.0%	-	-

LOD < 10 CFU/ml

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Table B5. Distribution of Total Coliforms Levels in Egg White Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	99	17.3%	99	17.3%
10 - 100	270	47.1%	369	64.4%
101-1,000	171	29.8%	540	94.2%
1,001-10,000	23	4.0%	563	98.3%
10,001-100,000	8	1.4%	571	99.7%
100,001-1,000,000	2	0.3%	573	100.0%
> 1,000,000	0	0.0%	573	100.0%
Total	573	100.0%	-	-

LOD < 10 CFU/ml

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Table B6. Distribution of Generic Escherichia Levels in Egg White Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
< LOD	277	48.3%	277	48.3%
10 -100	249	43.5%	526	91.8%
101-1,000	40	7.0%	566	98.8%
1,001-10,000	7	1.2%	573	100.0%
10,001-100,000	0	0.0%	573	100.0%
100,001-1,000,000	0	0.0%	573	100.0%
> 1,000,000	0	0.0%	573	100.0%
Total	573	100.0%	-	-

LOD < 10 CFU/ml

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Table B7. *Salmonella* Serotypes for Egg White

***Salmonella* Serotypes**		
Serotype Name	**Count**	**Percentage**
Braenderup	10	20.8%
Kentucky	10	20.8%
Heidelberg	7	14.6%
Enteritidis	4	8.3%
Mbandaka	4	8.3%
Thompson	4	8.3%
Cerro	3	6.3%
6,7:-:1,5,	1	2.1%
Miami	1	2.1%
Multiple Serotypes	4	8.3%
Total	**48**	**100%**

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Table B8. Statistical Comparison between Shift 1 and Shift 2 Egg White Samples from 22 Establishments

Indicators Pathogen	Sample at Shift 1	Mean at Shift 1	Mean St Error at Shift 1	Geo Mean at Shift 1	Log10 of Geo Mean at Shift 1	Sample at Shift 2	Mean at Shift 2	Mean Sd Error at Shift 2	Geo Mean at Shift 2	Log10 Geo Mean at Shift 2	p-value (*)
Generic *E. coli* (CFU/ml)	228	106	44	12.99	1.11	110	111	75	12.88	1.11	p = 0.90
APC (CFU/ml)	228	53,003	14,255	3,438	3.53	110	407,510	299,780	3,566	3.55	p = 0.97
Salmonella (MPN/ml)	25	5.28	1.37	0.60	-0.22	12	2.67	1.66	0.13	-0.88	p = 0.11
Salmonella (Percent Positive)		10.90%					10.90%				p = 0.98**

(*) Test based on Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums) at α = 0.05 between shift 1 and shift 2 for actual values (22 establishments with 2 shifts)
(**) Test of Proportion for percent positive

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Table B9. Nonresponse Rate and Percent Positive by Strata for Samples in Egg White

Strata	Discarded Samples	Valid Samples	Total Samples	Percent of Discarded	Percent Positive by Strata for *Salmonella*
1	75	160	235	31.9%	7.5%
2	4	310	314	1.3%	7.4%
3	103	103	206	50.0%	12.6%
Total/p-value	182	573	755	P= 0.000*	P = 0.22**

(*) p = 0.000 There is significant difference in percent discarded samples by strata.
(**) p = 0.22 There is no significant difference in *Salmonella* percent positive by strata.

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Table B10. *Salmonella* Percent Positive per Month for Egg White

Month Collected	Samples Collected	Samples *Salmonella* Positive	Percent Positive per Month
January	45	4	8.9%
February	48	5	10.4%
March	48	2	4.2%
April	45	3	6.7%
May	52	5	9.6%
June	48	4	8.3%
July	43	3	7.0%
August	53	5	9.4%
September	46	2	4.3%
October	49	2	4.1%
November	46	9	19.6%
December	50	4	8.0%

There is no statistical difference among percent positive per month (Test for homogeneity of proportions Chi-squared, p = 0.40)

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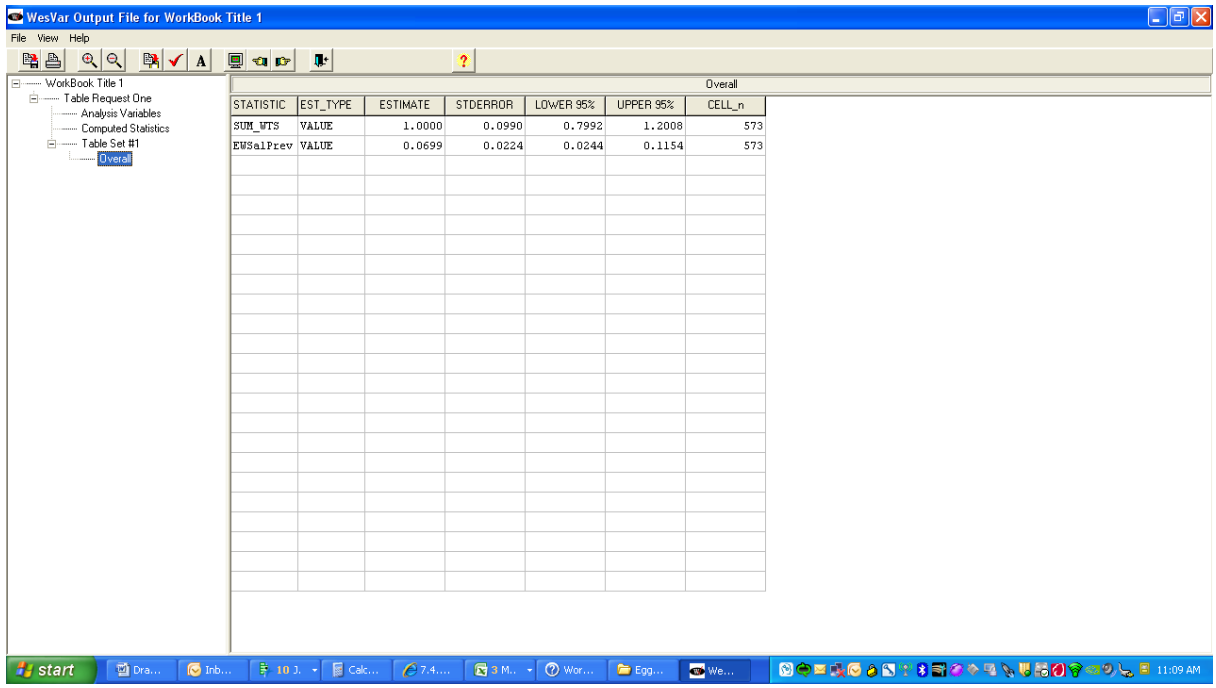
Figure 1B. WesVar Output Window for *Salmonella* Prevalence in Egg White



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SECTION C: EGG YOLK

Table C1. Summary for Quantified Egg Yolk Samples by Microorganism

Microorganisms ---------- Indicator Organisms [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Levels of Positives				
					Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count (APC)	**Pre-Pasteurization**	**554**	**539**	**97.29%**	**3.6×10^7**	**3.4×10^7**	**1,508**	**(1155 – 1968)**	**3.17**
Enterobacteriaceae	**Pre-Pasteurization**	**554**	**446**	**80.51%**	**1.37×10^6**	**1.37×10^6**	**99**	**(76 – 125)**	**1.99**
Total Coliforms	**Pre-Pasteurization**	**554**	**390**	**70.40%**	**2,500**	**1,272**	**39**	**(33 – 47)**	**1.59**
Generic Escherichia coli	**Pre-Pasteurization**	**554**	**233**	**42.06%**	**100**	**30**	**11**	**(10 – 12)**	**1.04**
Pathogenic Organism									
Salmonella [3]	**Pre-Pasteurization**	**554**	**145** [4]	**26.17%**	**6.35**	**1.17**	**0.67**	**(0.43 – 1.04)**	**-0.17**

(1) Units are CFU/ml
(2) Above Limit of Detection (LOD); LOD = 10 CFU/ml
(3) *Salmonella* measurements are in MPN/ml, LOD < 0.030 MPN/ml
(4) There are 28 positives under LOD in the MPN test; LOD < 0.030 MPN/ml.

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Table C2. Distribution of *Salmonella* MPN Levels in Egg Yolk Samples

Range, MPN/ml	Number of Samples[(1)]	Percent of Total	Cumulative Number	Cumulative Percent
< 0.030	28	19.3%	28	19.3%
0.030 - 0.30	32	22.1%	60	41.4%
0.301 - 3.00	33	22.8%	93	64.1%
3.01 - 30.00	50	34.5%	143	98.6%
> 30.01	2	1.4%	145	100.0%
Total	145	100.0%	-	-

LOD < 0.030 MPN/ml

(1) Only positive samples are included regardless if under LOD

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Table C3. Distribution of APC Levels in Egg Yolk Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	15	2.7%	15	2.7%
10 - 100	97	17.5%	112	20.2%
101 - 1,000	164	29.6%	276	49.8%
1,001-10,000	129	23.3%	405	73.1%
10,001-100,000	95	17.1%	500	90.3%
100,001-1,000,000	36	6.5%	536	96.8%
1,000,001-10,000,000	14	2.5%	550	99.3%
10,000,001-100,000,000	2	0.4%	552	99.6%
100,000,001-1,000,000,000	1	0.2%	553	99.8%
> 1,000,000,000	1	0.2%	554	100.0%
Total	554	100.0%	-	-

LOD < 10 CFU/ml

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Table C4. Distribution of Enterobacteriaceae Levels in Egg Yolk Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	108	19.5%	108	19.5%
10 - 100	207	37.4%	315	56.9%
101-1,000	142	25.6%	457	82.5%
1,001-10,000	66	11.9%	523	94.4%
10,001-100,000	23	4.2%	546	98.6%
100,001-1,000,000	7	1.3%	553	99.8%
> 1,000,000	1	0.2%	554	100.0%
Total	554	100.0%	-	-

LOD < 10 CFU/ml

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Table C5. Distribution of Total Coliforms Levels in Egg Yolk Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	164	29.6%	164	29.6%
10 - 100	223	40.3%	387	69.9%
101-1,000	123	22.2%	510	92.1%
1,001-10,000	35	6.3%	545	98.4%
10,001-100,000	6	1.1%	551	99.5%
100,001-1,000,000	3	0.5%	554	100.0%
> 1,000,000	0	0.0%	554	100.0%
Total	554	100.0%	-	-

LOD < 10 CFU/ml

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Table C6. Distribution of Generic *Escherichia* Levels in Egg Yolk Samples

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
< LOD	321	57.9%	321	57.9%
10 -100	193	34.8%	514	92.8%
101-1,000	26	4.7%	540	97.5%
1,001-10,000	13	2.3%	553	99.8%
10,001-100,000	1	0.2%	554	100.0%
100,001-1,000,000	0	0.0%	554	100.0%
> 1,000,000	0	0.0%	554	100.0%
Total	554	100.0%	-	-

LOD < 10 CFU/ml

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Table C7. *Salmonella* Serotypes for Egg Yolk

Salmonella Serotypes		
Serotype Name	**Count**	**Percentage**
Enteritidis	34	23.4%
Heidelberg	30	20.7%
Kentucky	28	19.3%
Braenderup	19	13.1%
Mbandaka	5	3.4%
Thompson	4	2.8%
Cerro	3	2.1%
Infantis	2	1.4%
Johannesburg	2	1.4%
Montevideo	2	1.4%
4,12:r:-	1	0.7%
4,5,12:i:-	1	0.7%
6,7:-:1,5,	1	0.7%
Alachua	1	0.7%
Miami	1	0.7%
Muenchen	1	0.7%
Rubislaw	1	0.7%
Tennessee	1	0.7%
Typhimurium var 5-	1	0.7%
Multiple Serotypes	7	4.8%
Total	**145**	**100%**

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Table C8. Statistical Comparison between Shift 1 and Shift 2 Egg Yolk Samples from 24 Establishments

Indicators Pathogen	Sample at Shift 1	Mean at Shift 1	Mean St Error at Shift 1	Geo Mean at Shift 1	Log10 of Geo Mean at Shift 1	Sample at Shift 2	Mean at Shift 2	Mean Sd Error at Shift 2	Geo Mean at Shift 2	Log10 Geo Mean at Shift 2	p-value (*)
Generic *E. coli* (CFU/ml)	197	98	22	13.61	1.13	118	268	135	12.55	1.09	p = 0.46
APC (CFU/ml)	197	130,967	87,012	1,446	3.16	118	7,577,454	7,542,086	1,813	3.25	p = 0.55
Salmonella (MPN/ml)	71	7.74	1.92	0.92	-0.03	44	6.49	2.20	0.77	-0.11	p = 0.66
Salmonella (Percent Positive)		36.04%					37.28%				p = 0.82**

(*) Test based on Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums) at α = 0.05 between shift 1 and shift 2 for actual values (24 establishments with 2 shifts)
(**) Test of Proportion for percent positive

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Table C9. Nonresponse Rate and Percent Positive by Strata for Samples in Egg Yolk

Strata	Discarded Samples	Valid Samples	Total Samples	Percent of Discarded	Percent Positive by Strata for *Salmonella*
1	57	199	256	22.3%	28.1%
2	47	276	323	14.6%	28.2%
3	52	79	131	39.7%	13.9%
Total/p-value	156	554	710	P= 0.000*	P = 0.03**

(*) p = 0. 000. There is significant difference in percent discarded samples by strata.
(**) p = 0.03 There is significant difference in Salmonella percent positive by strata.

Table C10. *Salmonella* Percent Positive per Month for Egg Yolk

Month Collected	Samples Collected	Samples *Salmonella* Positive	Percent Positive per Month
January	48	8	16.67%
February	46	6	13.04%
March	45	13	28.89%
April	42	12	28.57%
May	41	16	39.02%
June	49	17	34.69%
July	42	11	26.19%
August	51	17	33.33%
September	51	16	31.37%
October	45	10	22.22%
November	45	12	26.67%
December	49	7	14.29%

There is no statistical difference among percent positive per month (Test for homogeneity of proportions Chi-squared, p = 0.08)

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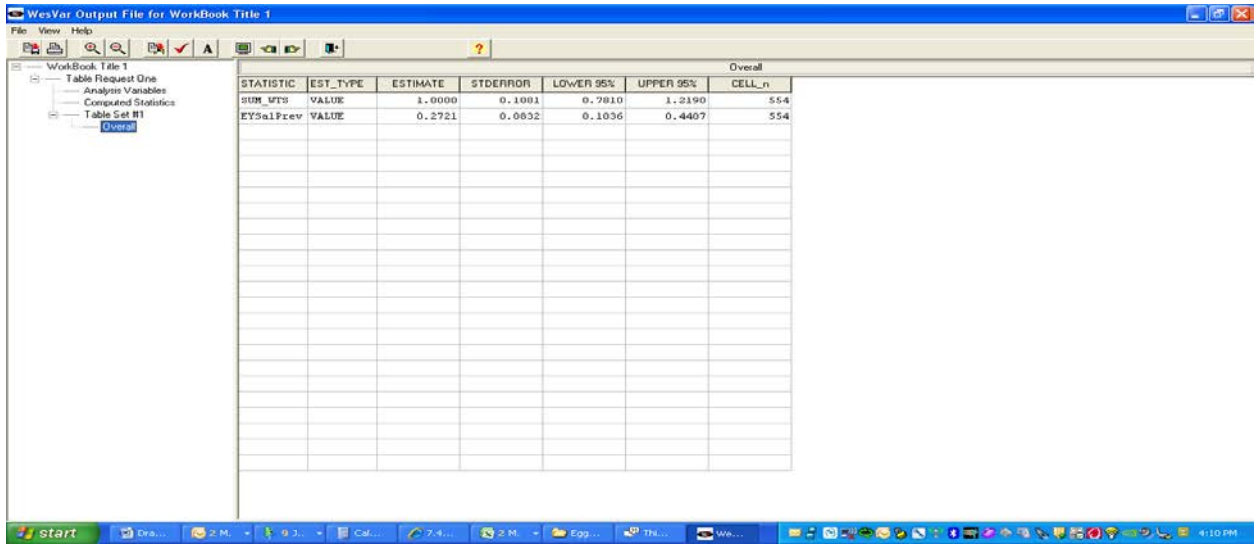
Figure C1. WesVar Output Window for *Salmonella* Prevalence in Egg Yolk



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APPENDIX

Statistical Analysis Methods

Work Flow Overview:

To calculate summary tables and national prevalence estimates, FSIS processed the data during and after the study in the following steps:

1. FSIS updated total production volume of raw liquid eggs throughout the 12 month survey period.

2. FSIS verified the final data set after completing the survey. Shipping date and collection date for each sample were checked to ensure they were no more than one day apart. The Agency confirmed that sample receipt temperatures were within analyzable limits and verified answers to supplemental questions on the sampling form. FSIS determined whether *Salmonella* had appropriate MPN values and MPN positive tube combinations. The laboratories obtained serogroup and serotype information for all *Salmonella* positive samples. FSIS identified outliers for indicator organisms and corrected data entry errors.

3. The analyst calculated general statistics, tested comparison hypothesis for pathogens and indicators, and assembled the results in tables.

4. The analyst merged existing files containing information about production volume to determine total production, calculated establishment and sample weight, and adjusted for nonresponses or missing samples. The analyst prepared sample files for special software processing. JMP (8) statistical software was used in the process.

5. FSIS analyzed sample files using the software "WesVar v 5.1" to obtain point estimates and uncertainty.

Data

The original raw data file for RLEBS contains answers to supplemental questions on the sample collection form, and lab results. In addition, a file with production volume for all establishments producing liquid egg during the 12-month survey was assembled. Volume information is essential for calculating the annual production and is used to calculate weight of each establishment. These files contain general information including:

a. Production volume information from all establishments that produced some kind of liquid egg during the survey.
b. A portion of establishments had no representation in the study because the establishments were not scheduled for sampling or all samples from specific establishments were discarded. Valid samples were collected on 47 establishments.
c. FSIS calculated additional information about stratification and production by stratum for each egg product.
d. The individual sample weight was calculated by integrating the production file with the survey results.
e. Other sections of the file showed establishment information including, establishment identification number, state, stratification calculations, and sample collectors' answers to questions posted on block 28 of the sample collection form
f. FSIS assembled a final file with valid results for calculation of the presence and concentration of microorganisms; 1,853 samples produced conclusive results.
g. For product-specific calculations the file was separated by liquid egg product
   1.- Whole eggs: 726 samples
   2.- Egg whites: 573 samples

3.- Egg yolks: 554 samples

Calculation of Base Sample Weights

The sample weights adjusting for production volume were applied to each sample results and to each product separately. The scope of the sampling design for the RLEBS divided the qualified producing establishments into three classes or strata specific to each egg product. Collecting an unequal number of samples from pre-determined groups implies that the sample collection is not completely random, so the establishments do not have an equal probability of selection (9). As such, some sectors of the population were sampled at a higher frequency, and this type of design can introduce bias. To counter-balance the bias, each sample is weighted to account for its relative impact on the result. A way to relate the sample results and their uncertainty to all of the establishments producing specific raw liquid eggs and to estimate parameters is by using special statistical software (discussed in "WesVar Statistical Procedures" section). However, before the application of the software the weight of each sample was calculated.

The base weight of a sampled unit is the reciprocal of its probability of selection into the sample (9). The weight acts as an equalizer representing the sampling units that were not selected. In mathematical notation, if a unit is included in the sample with probability *Pi,* then its base weight, denoted by W_i, is given by

$W_i = 1/P_i$

The base weights in the multi-stage RCPBS must reflect the probabilities of selection at each stage. In the case of a two-stage design, the *j-th* Primary Sampling Unit (PSU*,* the establishment*)* is selected with probability P_j at the first stage, and the *i-th* (lot of liquid egg) is selected with probability *pi(j)* at the second stage. Then the overall probability of selection of every unit in the sample is given by

$$P_{ij} = P_j * P_{i(j)}$$

And the base weight is the reciprocal

$$W_i = 1/P_{ij}$$

In case of a simple non-stratified sample, the weight (in relation to production volume) is $V_i / \Sigma V_i$ or volume of establishment *"i"* divided by total production (all establishments). In case of a two-stage stratified survey (like the row liquid eggs), each stratum is treated as an independent sample, and the basic weight of a establishment (PSU) in stratum *"j"* is

$$W_p = (V_j / \Sigma V_{sj}) * (V_{ij} / \Sigma V_j)$$

Where:

V_j is the volume of stratum *j* <u>including establishments not sampled</u>
$\sum V_{sj}$ is the volume of establishments that were <u>sampled</u> in stratum *"j"*
V_{ij} is the volume of establishment "*i*" in stratum "*j*"
Σv_j is total volume of establishments in the frame, <u>sampled or not</u>

This is the weight of the stratum times the weight of the establishment. Because the study's design calls for multiple samples drawn from individual establishments, the greater the number of samples taken from a establishment is, the smaller the individual sample weight for that establishment. As such, samples take shares of the weight of the establishment. In view of this fact, the weight for an individual sample is:

$$W_{ij} = 1/n_{ij} * (V_j / \Sigma V_{sj}) * (V_{ij} / \Sigma V_j) \qquad (1)$$

Where:

n_{ij} is the number of samples taken in establishment *"i"* in stratum *"j"*

Corrections for Nonresponses

It is rarely the case that all desired information is obtained from all sampled units. For instance, some samples may be discarded because of temperature deviations, or the establishment was not producing the product at collection time. This type of missing information is called unit nonresponse and may create bias in the estimate (10, 11).

If there are systematic differences (non-random) between the respondents and non-respondents among strata, then estimates based solely on the respondents may be biased.
The size of the nonresponse bias for a sample mean is a function of two factors:

- The proportion of the population that does not respond by strata.
- The size of the difference in population means among strata.

If the proportions of response in the strata are not significantly different, then there is no need for adjustment, because the same proportion of samples is missing in each stratum. If the population mean of interest has no significant difference among strata, there is no need for adjustment. If one of the above conditions is not satisfied, then there is a need to adjust for nonresponse.

Essentially, the adjustment for nonresponse transfers the base weights, previously calculated, of all eligible non-responding sampled units to the responding units. This transfer is implemented in the following steps:

1. Compute the response rates for each stratum;
2. Use the reciprocal of the stratum response rates for nonresponse adjustments; and
3. Calculate the nonresponse adjusted weight for the *j-th* establishment as:

$W_j = W_{1j} * W_{2j}$

Where:
W_{1i} is the base weight (formula 1)
W_{2i} is the nonresponse adjustment

Adjustment for Nonresponse for Liquid Egg Products

Whole Eggs
Table A9 provides a summary of the response rate per stratum and the percent positive of *Salmonella* per stratum. Stratum 3 shows a disproportionally and statistically significant (p = 0.04) higher number of missing samples, and stratum 3 also shows at p= 0.005 a significantly lower percent positive of *Salmonella*. This indicates that there is a need for adjustment for nonresponse for whole egg samples.

Egg white
Table B9 provides a summary of the response rate per stratum and the percent positive of *Salmonella* per stratum. Stratum 2 shows a disproportionally and statistically significant (p = 0.000) lower number of missing samples. There is no significant differences of percent positive of *Salmonella* at p= 0.22. Because the response rate by stratum is significant, there is a need for adjustment for nonresponse for egg white samples.

Egg Yolk
Table C9 provides a summary of the response rate per stratum and the percent positive of *Salmonella* per stratum. Stratum 2 shows a disproportionally and statistically significant (p = 0.000) lower number of missing samples. In addition, there is significant differences of percent positive of *Salmonella* at p= 0.03. This indicates that there is a need for adjustment for nonresponse for egg yolk samples.

The adjusted weight for each product was calculated with the information in the data files and variables developed using previously defined formulas. After additional preparation, the analyst assembled a WesVar software ready–to-use file for each product and ran the software.

Furthermore, because the survey was conducted for an entire year, FSIS is interested in finding *Salmonella* seasonality (per month differences in percent positive) during the survey. A test of multiple proportions (Chi-squared) by month (12) was done for each product. Tables for each product showing the percent positive per month were assembled and test calculated for each product. At a level of confidence of 0.05, there are no percent positive significant differences by month for *Salmonella* for any product. Tables and results for each product follow.

Whole Eggs

Table A10 shows percent positive of *Salmonella* by month for whole egg samples. The Test for homogeneity of proportions at Chi-squared p = 0.46 indicates that there is no statistical difference among percent positive by month.

Egg white

Table B10 shows percent positive of Salmonella by month for egg white samples. The Test for homogeneity of proportions at Chi-squared p = 0.40 indicates that there is no statistical difference among percent positive by month.

Egg Yolk

Table C10 shows percent positive of *Salmonella* by month for egg yolk samples. The Test for homogeneity of proportions at Chi-squared p = 0.08 indicates that there is no statistical difference among percent positive per month. However, there is a tendency for colder months to have a lower *Salmonella* percent positive.

WesVar Statistical Procedures

When data are collected as part of a complex sample survey, analytically there is often no easy way to produce unbiased design-consistent estimates of variance. The variances of survey statistics, including means and proportions that are estimated using standard statistical packages, are usually inappropriate and are often too small. Replication methods provides a method to estimate variance for the types of complex sample designs and weighting procedures like the one encountered in this study.

The basic idea behind replication is to select subsamples repeatedly from the whole sample, calculate the statistics of interest for each subsample, and then use these subsamples or replicates to estimate the variance of the full sample statistics. The subsamples are called replicates and the statistics calculated from these replicates are called replicate estimates. Because of the weighting and the application of the replication method, the outcome obtained in the sampling can be extended to the entire U.S. operation as a national prevalence measurement. The replication methods and theory used in this survey derive from the computer statistical package WesVar version 5.1. The package provides several methods of replication, including the Balance Repeated Replication (BRR) and the Jackknife procedures (JKs). For the particular design of the sample at hand with many establishments or Primary Sampling Unit (PSU) per stratum, the methodology selected was the Jack Knife (n) (13).

One of the main advantages of replication is its ease of use at the analysis stage. The same estimation procedure is used for the full sample and for each replicate. The variance estimates are then readily computed by a simple procedure. Furthermore, the same procedure is applicable to most statistics, such as means, percentages, ratios, correlations, etc. These estimates can be calculated for analytic groups or sub-populations. Another important advantage of replication is that it provides a simple way to account for adjustments that are made in weighting (14, 15, 16).

WesVar accomplishes the implementation of the replication methods in four steps.

Step 1 WesVar divides the sample into subsample replicates that mirror the design of the sample by specifying the variance of the variables strata and PSU.

Step 2 WesVar calculates weights for each replicate, following the same procedures used for the full-sample weight. The replicate weights are attached to the WesVar data file.

Step 3 The software calculates replicate estimates for each of the replicates using the same methods used for the full sample estimate.

Step 4 WesVar estimates the variance of the full-sample estimate, using the resulting full-sample and replicate estimates. The outputs of the program reflect this computation.

The next step calculates the replicated weights. The WesVar program accomplished this by using the variables strata, already in file, (the division of establishments by size, 1 to 3) and a new variable PSU. The analyst created the variable PSU (establishments) by allocating a number (1 to n) to each PSU in each stratum; this allowed for the partition of the sample into subsample replicates that mirrored the design of the sample. With the introduction of the variables weights, strata, and PSU, the file was finally ready for processing in WesVar.

Calculation of *Salmonella* National Prevalence for Raw Liquid Eggs

Each individual product data-file was submitted to the WesVar software. The software window output with results for *Salmonella* for each product is presented in figures A1, B1, and C1. Because of the use of replicated weight, these results extend to the entire universe of liquid eggs in the U.S. market.

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UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
WASHINGTON, DC

FSIS NOTICE	16-12	2-29-12

NATIONWIDE RAW LIQUID EGG MICROBIOLOGICAL BASELINE SURVEY – UPDATE

NOTE: DO NOT IMPLEMENT THIS NOTICE UNTIL MARCH 12, 2012

I. PURPOSE

This notice announces that the Food Safety and Inspection Service's (FSIS) Nationwide Raw Liquid Egg Microbiological Baseline Survey (RLEBS) will begin on March 12, 2012, and that the 90-day training ("shake down") period referred to in FSIS Notice 52-11, nationwide FSIS RLEBS – Shakedown, has ended.

NOTE: This notice applies only to inspection program personnel (IPP) at plants that are included in the nationwide FSIS RLEBS. Only plants that pasteurize or heat treat dried egg products are included.

KEY POINTS

- *FSIS Notice 52-11 is cancelled.*
- *Only egg products plants that pasteurize or heat treat dried egg products, will be included in the baseline survey. Egg products plants that only break shell eggs will not be included in the baseline survey.*
- *The sample container to be used has been changed to ensure a more secure seal and prevent leakage during sample shipment.*
- *Samples are to be collected on both shifts in multiple shift establishments.*
- *Each sample will consist of one 400 ml sample. The sample is to be collected prior to the addition of any ingredients and prior to pasteurization or heat treatment, which may require the sample to be collected the day before pasteurization.*
- *When the lot of egg product to be sampled is to be dried, the sample is to be*

DISTRIBUTION: Electronic	NOTICE EXPIRES: 3/1/13	OPI: OPPD

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